REMARKS

Claim 4 is amended herewith to clarify the claimed invention and to sharpen its definition over the prior art of record.

In particular, claim 4 as filed recited in step a) that the DNA vector containing the nucleic acid of interest "and replicating in said prokaryotic or eukaryotic cell" is contacted with a mutagenic agent blocking the DNA replication in the cell. The quoted language was not intended to suggest a replicating step in addition to the recited contacting step; rather, the quoted language was intended to recite the property of the recited DNA vectors of being replication-competent in the prokaryotic or eukaryotic cell containing the target sequence (at least prior to treatment of the DNA vector with the mutagenic agent). That interpretation is consistent with the disclosure of the specification, see, e.g., the examples.

The present amendment clarifies the language of claim 4 in this respect by now reciting that the DNA vector containing the nucleic acid of interest is "replication competent in said prokaryotic or eukaryotic cell" and is contacted with a mutagenic agent blocking the DNA replication in the cell. As noted above, it is apparent from the examples in the specification that this is the proper interpretation of the original claim language, and it is moreover supported elsewhere throughout the specification,

see, e.g., the reference to a "replicative vector" at p. 6, line 25 and the phrasing "and which replicates in said prokaryotic or eukaryotic cell" at p. 7, line 4. See also page 10, lines 11-18 and 28-32, where it is described that the nucleic acid of interest comprises an *E. coli* replication origin that is used to transform *E. coli* cells. Likewise in the eukaryotic model (p. 22, lines 3-8), the nucleic acid of interest comprises an Epstein-Barr replication origin which allows this nucleic acid of interest to replicate a number of times in a human eukaryotic cell transformed by this nucleic acid molecule.

With the language of claim 4 thus clarified, its patentability relative to the previously-applied prior art is believed to be more readily apparent.

In particular, as to the rejection of claims 4-6, 10-14, 16-18 and 21-22 under 35 USC §102(b) as anticipated by HINDS et al., the DNA vector of the present claims is replication competent in the target cell prior to treatment with the mutagenic agent, whereas the suicide plasma of HINDS by design is not. Indeed, the present specification describes the suicide plasma technique of HINDS as having an undesirably low rate of homologous recombination, which is greatly improved by the different technique of the present invention.

In greater detail, the DNA molecules described by HINDS, namely, suicide plasmids, single stranded DNA, and

phagemids, are all replication-incompetent. A suicide plasmid is one which, due to a number of different mechanisms, cannot replicate in a host cell following transfection and which comprises a selectable marker gene. This means that selection of cells transfected with the suicide plasmid results in all selected clones having a genomic insertion of the selectable marker gene and associated sequences.

A phagemid or phasmid is a type of cloning vector developed as a co-infection of the M13 helper phage and plasmids to produce a smaller version of the virus. Phagemids contain an origin for double stranded replication as well as an origin for single stranded replication, mostly not comprising the entire phagemid (*i.e.*, only a small part of the phagemid is copied as a single strand), but not all the necessary viral genes to replicate. This means that the presence of a "helper" virus such as f1, is required to provide the rest of the genes necessary to replicate viral proteins to thereby allow more virus particles to be created using the host cell's resources. Without this "helper" virus, however, the phagemid is incapable of independent replication.

As to the rejection of claims 4-5, 10-14, 16-19, 21 and 23 under 35 USC §102(b) as anticipated by GANIATSAS, the heat shock or UV exposure described in that publication is performed after the alteration of the genome of the ES cell line and the

selection and propagation of the altered cell line to a sufficient density so as to allow western blot analysis to be performed.

By contrast, in claim 4 the treatment of the DNA vector with the mutagenic agent occurs before the cell containing the target sequence is transfected. Claim 4 as amended herewith also now makes explicit that the step c) selection of prokaryotic or eukaryotic cells for which the nucleic acid of interest has been integrated into the predetermined target nucleotide sequence, is performed on the prokaryotic or eukaryotic cells that contain the nucleic acid of interest as a result of the transfection of step b).

As GANIATSAS does not disclose at least these aspects of claim 4, it follows that none of claims 4-5, 10-14, 16-19, 21 and 23 is anticipated by that reference.

Lastly, as to the rejection of claims 4-21 and 23-24 under 35 USC §102(e) as anticipated by HOEIJMAKERS, the exposure of the altered and unaltered cells of that reference to a DNA lesion inducing agent such as UV, occurs only once suitably and stably transformed cells have been created, selected and propagated into whole animals from which further samples can be derived.

Again, claim 4 differs from the disclosure of HOEIJMAKERS in its recitation that the DNA vector containing the

nucleotide sequence of interest is treated with a mutagenic agent prior to its use in the transformation of a target prokaryotic or eukaryotic cell. As HOEIJMAKERS does not disclose at least this aspect of claim 4, it follows that none of claims 4-21 and 23-24 is anticipated by that reference.

In view of the present amendment and the foregoing remarks, it is believed that this application is now in condition for allowance with claims 4-24, as amended. Allowance and passage to issue on that basis are accordingly respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

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